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Research article

Plastic and genetic responses to shifts in snowmelt time affects the reproductive phenology and growth of *Ranunculus acris*☆

Nicola Delnevo[a,1], Alessandro Petraglia[b], Michele Carbognani[b], Vigdis Vandvik[a], Aud H. Halbritter[a,*]

[a] Department of Biology, University of Bergen, POBox 7803, 5020 Bergen, Norway
[b] Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, 11/A Parco Area delle Scienze, 43124 Parma, Italy

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ABSTRACT

Changes in both temperature and precipitation will affect snowmelt time at high elevation, thereby influencing plant reproduction and growth. Species can respond to changed climate with phenotypic plasticity or genetic adaptation, and these responses might vary at different levels of advanced and delayed snowmelt time. Here we mimicked future climate change projections for western Norway by transplanting individuals of *Ranunculus acris* towards warmer, wetter and warmer & wetter climates. And we replicated the experiment along regional-scale temperature and precipitation gradients. This setup resulted in both advanced (warmer and warmer & wetter transplants) and delayed (wetter transplants) snowmelt in the experimental sites. We recorded phenological development and growth over one growing season.

The reproductive phenology of the transplanted *R. acris* individuals was affected by both phenotypic plasticity and genetic differences between populations of different origins, while growth showed only plastic responses. Plants expressed high plasticity to both advanced and delayed snowmelt time by acceleration of the onset of buds, flowers and fruits. Only the plants from wet and high-elevation sites showed a small response to advanced SMT. The late snowmelt time these populations experience could potentially cause high selection pressure leading to more constrains in plasticity. When grown under common conditions, plants from late snowmelt sites responded with earlier onset of phenological development, suggesting that the timing of snowmelt exerts strong selection on reproduction. To project species fates under future climate we need to consider the interplay between genetic adaptation and plastic responses under different climate contexts, especially towards the species range limits.

1. Introduction

Arctic and alpine ecosystems are characterized by seasonal snow cover, which protects plants against low temperature, desiccation, and ice blast during the cold season, and which also determines the duration of the snow-free period and thus defines the time available for growth and reproduction (Bliss, 1962; Jonas et al., 2008; Körner, 2003; Larcher, 2003). The snowmelt time (hereafter SMT) is regarded one of the most important drivers for growth and reproduction at high latitudes and in the mountains, affecting community composition and distribution (e.g. Braun-Blanquet, 1932; Gjærevoll, 1956; Scott and Billings, 1964). Climate change, such as increased temperature and changes in precipitation patterns, is likely to alter the timing of snowmelt, and hence the time available for plants to grow and reproduce, which can have both immediate and long-term consequences for species composition and distribution (Cooper et al., 2011; Heegaard, 2002).

The combination of temperature and precipitation influence the SMT in arctic and alpine ecosystems in complex ways. Winter accumulation of snow defines the amount of snow that has to melt in spring, while spring temperature affect the rate of snowmelt. Current climate projections predict changes in the mean and variances of both temperature and precipitation (IPCC, 2014), especially for alpine environments at high latitudes (AICA, 2004; Stocker, 2013). Climate projections for the oceanic regions in north-western Europe indicate a

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* Corresponding author.
E-mail addresses: n.delnevo@ecu.edu.au (N. Delnevo), alessandro.petraglia@unipr.it (A. Petraglia), michele.carbognani@unipr.it (M. Carbognani), vigdis.vandvik@uib.no (V. Vandvik), aud.halbritter@uib.no (A.H. Halbritter).
[1] Present address: School of Science, Edith Cowan University, 270 Joondalup Dr, 6027 Joondalup, WA, Australia.

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warming rate greater than the global average coupled with marked increases in precipitation and in the snow-to-rain ratio (Hanssen-Bauer et al., 2009). These predicted increases in both temperature and snowfall may have opposing effects on SMT, with increased snowfall delaying and higher spring temperature advancing SMT. (Bjorkman et al., 2015). These changes also affect the thermal conditions experienced by plants (Jonas et al., 2008). Indeed, earlier SMT due to warming may expose plants to colder conditions and increase the risk of frost events (e.g. Inouye, 2008), later SMT due to increased snow accumulation in winter provides plants with a shorter, but warmer growing season, as long as the season does not become too short (Carbognani et al., 2016). Consequently, given the complex interplay among the climatic factors, it is challenging to predict how plants will respond to these concurrent climatic changes, and especially their climatic tolerance limits under novel climatic conditions (Bjorkman et al., 2015; Donnelly et al., 2012; Franks et al., 2014).

Plant populations' abilities to respond to fluctuations and change in SMT in space and time is limited by their phenotypic plasticity, genetic adaptations, or a combination of both (Donnelly et al., 2012; Forrest and Miller-Rushing, 2010; Franks et al., 2014). In the short-term, phenotypic plasticity may allow plants to counter the detrimental effects of changing environmental conditions and buffer populations against climate fluctuations (Nicotra et al., 2010). In the long-term population adaptive potential is key for species persistence and community stability (Byars and Hoffmann, 2009). Numerous studies have investigated the plastic response of growth and reproduction to changes in SMT in arctic and alpine habitats (Carbognani et al., 2012; Cooper et al., 2011; e.g. Galen and Stanton, 1995; Heegaard, 2002; Hülber et al., 2010; Petraglia et al., 2014; Totland and Alatalo, 2002). Generally, advanced SMT prolongs the pre-flowering period, while delayed SMT accelerates the phenological development, whereas growth responses are very variable (reviewed in Wipf and Rixen, 2010). However, responses vary among species (Cooper et al., 2011; Totland and Alatalo, 2002; Wipf et al., 2009) and with site-specific factors (Carbognani et al., 2016), for example soil moisture (Gimenez-Benavides et al., 2007; Winkler et al., 2016). Responses to changes in SMT can also be affected by local adaptations exerted from changes in selection pressure along climate gradients. Common garden and reciprocal transplant experiments, the classical approach to disentangle phenotypic plasticity and genetic differentiation (Merilä and Hendry, 2014), provide evidence for adaptation in reproductive phenology to SMT (Haggerty and Galloway, 2011; Hall and Willis, 2006; Kawai and Kudo, 2011; Stinson, 2004). Early-flowering plants might be at a disadvantage in areas where snow melts early, due to risk for late-frost events early in the season (Thomson, 2010). In late snowmelt habitats, on the other hand, flowering early might give a selective advantage, allowing to complete the life cycle during the short growing season (Anderson et al., 2012; Stinson, 2004). However, the evidence of such adaptive responses to SMT are less frequent (Donnelly et al., 2012; Frei et al., 2014; Stanton and Galen, 1997), and typically species-specific (Byars and Hoffmann, 2009).

Although some patterns in species responses to shift in SMT are emerging, the consistently high species- and site-specific variation begs the question of both the generality of the patterns and of the underlying drivers of the variation. One way to address this challenge is to explicitly and systematically address if and how responses vary across species and sites, e.g., by replicating the same experiments along environmental gradients (Dunne et al., 2003), an approach also known as "distributed experiments" (Borer et al., 2014).

This study was conducted in south-western Norway, a fjord landscape characterized by strong regional-scale climate gradients: a precipitation gradient from the continental east to the oceanic west coast, and a temperature gradient from the temperate, boreal sea-level to alpine climates at high elevation. In this region, the combination of the two gradients result in early SMT at drier and low-elevation sites, and late SMT at wet and high-elevation sites (Fig. 1). We replicated our experiments across these two gradients to explore how the climate context, as reflected in variation in local SMT, affects reproductive phenology and growth of an alpine forb. Across these sites, we conducted a transplant experiment to explore how populations originating from different climatic contexts will respond to climate change. Since the climate change projections for our region predict increased temperatures and increased precipitation, we transplanted individual plants of our study species, *Ranunculus acris* towards warmer sites, wetter sites and warmer & wetter sites. We recorded phenological response and growth of the transplanted individuals compared to local populations in the origin site and in the transplanted localities over one growing season. Specifically, we ask: (1) does *R. acris* respond to a change SMT in reproductive phenology and growth? And (2) to what extent are such plastic responses in the reproductive phenology and growth of *R. acris* limited by genetic differences between populations? We expect plants to accelerate phenology (i.e. flower faster after SMT) both when transplanted to sites with advanced SMT due to warming and when transplanted to sites with delayed SMT due to increased snowfall. This is because phenology is affected by temperature, and we expect higher temperatures both at low-elevation sites and later in the season under the same temperature regime. Further, earlier phenology is expected in plants originating from sites with late SMT, as these populations experience strong selection for earlier flowering (Anderson et al., 2012). The predictions for the combined effect of increased temperature and increased precipitation are less clear, because the effects could cancel each other out, or be additive or be multiplicative. Finally, we ask (3) how do these responses in reproductive phenology and growth vary along the climate gradients? We expect plants from harsher conditions, i.e. wet and high-elevation sites, to be more strongly genetically determined in their response to SMT and therefore more constrained in plasticity (Gugger et al., 2015; Vitasse et al., 2013). And we expect the magnitude of response to vary with the magnitude in SMT (Wipf and Rixen, 2010).

2. Material and methods

2.1. Study sites and species

The study was conducted in south-western Norway within semi-natural grassland sites selected to represent a 'climate grid' of sites along broad-scale temperature (three levels, ca. 2 °C apart) and precipitation (four levels ca. 700 mm apart) gradients (Klanderud et al., 2015; Meineri et al., 2014). We selected four sites within the climate grid to represent contrasting climatic contexts (Fig. 1, Table 1); two temperature levels (ca. 7.5 and 9.5 °C mean temperature of the four warmest months), each combined with two precipitation levels (ca. 2000 and 2700 mm annual precipitation). The climate data are interpolated with 100 m resolution from the normal period 1961–1990 (Tveito et al., 2005). All sites are grasslands on calcareous bedrock and were specifically selected to be similar in slope, exposure, grazing pressure, soils, and other ecologically relevant variables, so as to maximize impacts of the regional climate differences (Klanderud et al., 2015). To avoid animal interference with the experimental treatments and the transplanted plants, the experimental areas were fenced during the growing season. At each site, temperature was measured continuously at 30 cm above the ground using UTL-3 version 3.0 temperature loggers (GEOTEST AG, Zollikofen, Switzerland).

SMT advanced along the temperature gradient and was delayed with higher precipitation, with earlier SMT at low-elevation and drier sites (Fig. 1b and c). In 2015, the snow melted in the second half of June in the low-elevation sites and between early July and mid-August at the high-elevation sites (Fig. 1c, Table 1); SMT was 17–50 days earlier at the low-elevation sites and 7–40 days later in wetter sites. Mean daily temperature in the first week after snowmelt was up to 2 °C warmer in the high compared to the low-elevation sites.

Ranunculus acris (Ranunculaceae) is a common perennial herb with a circumpolar distribution; in south-western Norway it grows in a wide range of habitats, but is most abundant on medium dry meadows. *R. acris* flowers are self-incompatible (Totland, 1994). It is an early flowering species and leaves begin to develop precociously after snowmelt

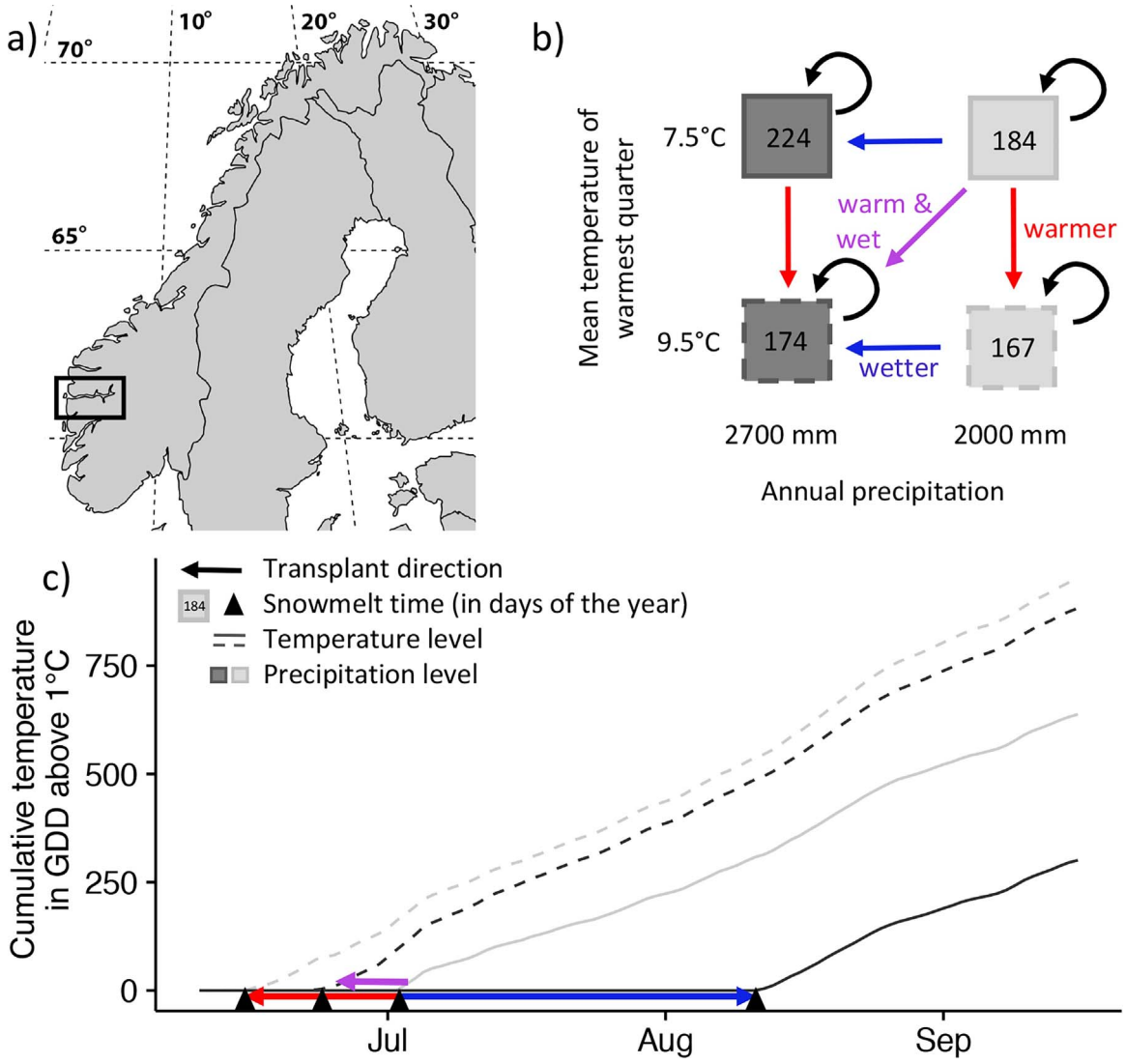

Fig. 1. a) Map of the location of the transplant sites in south-western Norway and b) the transplant design. The four sites shown by boxes, are located in a climate grid representing different temperatures (line type) and precipitation levels (grey shading). The number inside the box indicates the snowmelt date in days of the year. Arrows show the direction of transplant from the origin site (arrow tail) to the destination site (arrow tip) for the warmer (red), wetter (blue) and warmer & wetter transplant treatment (purple) and the local controls (black). Some of the sites thus represent both origin and destination sites. (c) Cumulative temperature (sum of mean daily temperature above 1 °C after snowmelt). The black triangles indicate the timing of snowmelt at each site. The arrows show the direction of transplant from the dry high-elevation site. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Geographic and climatic features of the four transplant sites (according to their temperature and precipitation level); coordinates, elevation, mean annual precipitation, mean temperature of the four warmest months (interpolated with 100 m resolution from the normal period 1961–1990, Tveito et al 2005), snowmelt date in 2015 in day of the year (DOY) and mean daily temperature in the first week after snowmelt are shown.

Temperature level (Elevation) Precipitation level	Cold High dry	Cold High wet	Warm Low dry	Warm Low wet
Latitude (°N)	60.83	60.93	61.09	60.54
Longitude (°E)	7.18	6.41	6.63	6.51
Elevation (m a.s.l.)	1213	1088	769	797
Precipitation (mm)	1925	2725	1848	3029
Temperature (°C)	5.87	6.58	8.77	8.67
Snowmelt date (DOY)	184	224	167	174
Temperature (°C) first week	9.7	9.1	8.5	7.1

and initiate the reproductive development early in the season (Hegland and Totland, 2007). Typically, individuals of high-elevation populations produce a single flower (Totland, 1997). We chose this species because it is one of the most common perennial forbs in the study system, it is dependent on pollinators and well studied (Totland, 1997, 1994, 2001).

2.2. Transplant experiment

In September 2014, at the end of the growing season, individual plants of approximately the same size (6.34 ± 3.07 cm) were collected within populations at the four sites. Each plant was carefully excavated from the ground, surrounded by a soil core of 10 cm in width and 10 cm in depth, to ensure that the roots were not damaged. Plants were randomly allocated to four transplant treatments, representing the expected change in climate: (i) warmer (Fig. 1b, red arrow), (ii) wetter (blue arrow), and (iii) warmer & wetter climate (purple arrow), and a local control (black arrow). Controls were planted back into another location at the original site to control for the transplant effect *per se*. Plants were only transplanted in one direction (not reciprocally), because we were mainly interested in understanding the effects of the projected climate change in the region.

From each site and treatment 30–40 plants were transplanted, adding up to a total of 269 *R. acris* individuals for the entire experiment (see Appendix A for the number of individual plants per transplant site and origin). Fewer plants were transplanted in the wetter transplant treatment at low-elevation sites than originally designed because we were unable to locate enough plants at the dry low-elevation site. Each plant was individually labelled, with labels placed so that they did not shade the plants or interfere with growth.

2.3. Data collection

Phenological observations started in 2015 after SMT and were carried out weekly until mid September, when the plants started to senescence. In these surveys, the phenophases for each target plant was recorded: budding (pedicellate buds emerging from basal rosette on a stalk), flowering (anthesis with completely open flowers) and fruiting (expanding fruits without flowering structures). During the 2015 growing season, 18 plants produced more than one flower, but we considered only the first bud the plant produced. In *R. acris* the different phenophases have limited overlap within individuals and if produced, the second bud appears after the first one is flowering and flowers when the first one is in fruiting (Totland, 1997, 2001). At each site, 6–15 such phenological observations were carried out, depending on the length of the snow-free period. Growth of each individual was assessed by measuring the longest leaf of the rosette at the end of the growing season 2015.

2.4. Data analyses

A generalized linear model was fitted independently for onset of bud, flower and fruits, expressed in days after snowmelt or leaf size as response variables. To account for non-normal distribution of residuals and non-homogeneous variances, we use a Poisson error distributions (appropriate for count data) for the phenological variables and a Gamma error distribution (appropriate for continuous positive data) for leaf size and checked that the assumptions were fulfilled by visual inspection of residual patterns (Zuur et al., 2009). We fitted a separate model for each transplant treatment (warmer, wetter and warmer & wetter), because the transplants are compared to different origin- and destination-control plants, for the different treatments (Fig. 1). Each model contained one transplant treatment and precipitation level (dry vs. wet for warmer treatment) or temperature level (low vs. high elevation for wetter treatment) to define the position in the climate grid and their interaction as predictors. The combined warmer & wetter transplant treatment had no interaction term, because this treatment was replicated only once.

To test for plasticity in the onset of phenology and plant growth, the plants in each transplant treatment were compared to the local control plants from the site of origin (hereafter origin-control). Whereas to test for genetic differentiation between populations in the phenological development and plant growth, the plants in each transplant treatment were compared to the local control plants from the destination site (hereafter destination-control). Only 3 individuals transplanted to wetter sites reached fruiting stage and this phenophase was therefore not analysed for plants transplanted to wetter climate. All the analyses were performed in R version 3.3.2 (R Development Core Team, 2016).

3. Results

3.1. Plastic response in reproductive phenology and growth

Plants in all transplant treatments produced buds, flowers and fruits consistently faster than the corresponding origin-control plants. Budding, flowering and fruiting was advanced on average by 7–11 days when transplanted to warmer climate, compared to the origin-controls (Fig. 2; Appendix B). Plants transplanted to wetter climate advanced the onset of budding and flowering by 9–12 days compared to the corresponding controls. And in the warmer & wetter transplant treatment, plants advanced the onset of each phenophase by 14–21 days relative to the origin-control.

The phenological development of *R. acris* also showed significant differences in relation to SMT. Plants advanced the onset of the phenophases significantly more at drier sites (i.e. where SMT was less advanced; Appendix B; precipitation level term for bud: $P < 0.001$, flower: $P < 0.001$ and fruit: $P < 0.001$) and the onset of flowering at high-elevation sites (i.e. where SMT was more delayed; temperature level term $P = 0.005$). The interactions between the transplant treatments and either precipitation or temperature levels were not significant, indicating consistent plastic responses in the onset of reproduction when transplanted to both warmer and wetter climate irrespective of the change in SMT. The only exception were plants transplanted to wetter climate, where bud swelling was more advanced in high-elevation than low-elevation sites (wetter treatment × temperature level: $P = 0.001$).

Ranunculus acris leaves also responded plastically to the transplant treatments. Plants had twice as large leaves in the warmer treatment and 1.4 times larger in the warmer & wetter treatment, compared to origin-control plants growing at high-elevation sites (Fig. 3 left side). In contrast, leaves of plants transplanted to wetter climate were 0.6-0.8 times smaller compared to the corresponding controls. The negative effect of transplanting to wetter climate on leaf growth was stronger at low compared to high-elevation sites (wetter treatment × temperature level: $P = 0.049$). These changes match patterns in leaf size of *R. acris* in relation to the temperature gradient, as plants at high elevation had significantly smaller leaves than those at low elevation (data not shown).

3.2. Genetic differences in reproductive phenology and growth

Plants transplanted towards warmer climates initiated all phenophases 7–15 days earlier than the local destination-control plants (Fig. 4; Appendix C). In contrast, plants transplanted towards wetter climates initiated budding and fruiting 8–10 days later than the destination-controls. For the warmer & wetter transplant treatment, plants did not differ in the onset of phenology compared to destination-control.

The onset in phenology differed also depending on the delay in SMT. The phenological development was significantly more delayed at low-elevation (i.e. where SMT was less delayed) compared to high-elevation sites (Appendix C; wetter treatment x temperature level for bud: $P = 0.012$; flower: $P = 0.022$). For example, budding was delayed by 15.0 days at low-elevation, but only by 1.1 days at high-elevation sites and flowering was delayed by 17.7 days in the low-, but only by 2.8 days at high-elevation sites.

Leaf size of *R. acris* did not differ from destination-controls under any transplant treatments (Fig. 3 right side, Appendix C). However, low-elevation plants decreased leaf size more compared to corresponding control plants when transplanted to wetter climate than high-elevation plants (wetter treatment × temperature level: $P = 0.015$). Leaf size also depended on the delay in SMT, with longer leaves in low-elevation compared to high-elevation sites (temperature level: $P < 0.001$).

4. Discussion

4.1. Plastic versus genetic responses in ranunculus acris

Ranunculus acris responded plastically in the velocity of the onset of reproductive phenology and growth to both advanced and delayed SMT. Plants transplanted to sites with advanced or delayed snowmelt were exposed to warmer temperature compared to the origin-control plants, which can explain the acceleration in the phenological

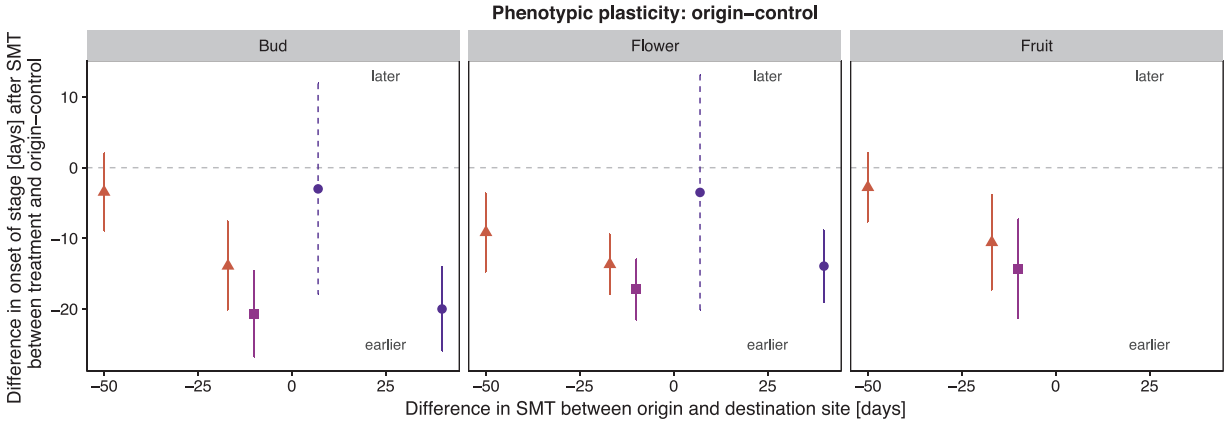

Fig. 2. Plasticity in phenological development as the difference in onset of a phenological stage in days since SMT ± 2SE between treatment and origin-control plants. The x-axis represents the difference in SMT between origin and destination site. The different stages are bud, flower and fruit. The colours and symbols indicate the transplant treatments to: warmer (red triangle), wetter (blue circle) and warmer & wetter (purple square) climate. Points below/above the dashed grey line indicate earlier/later days since SMT for onset in a stage in the transplanted plants compared to the origin-control plants. Closed circles indicate a significant difference between treatment and origin-control plants. Dashed error bars indicate a sample size lower than 6 individuals.

development (Carbognani et al., 2016; Dunne et al., 2003; Petraglia et al., 2014; Wipf and Rixen, 2010). Interestingly, plants in the warmer & wetter transplant treatment responded similarly in the onset of phenology as for the plant in the wetter treatment, but more strongly than the warmer treatment. This suggests that the effect of warmer & wetter conditions may not be fully additive. However, SMT advanced very similarly in both the warm treatment at the dry end of the climate grid and the warmer & wetter treatment and we therefore do not expect a large difference in the onset of phenology between these treatments. Also, leaf size increased in response to advanced SMT, while delayed SMT had a negative effect on growth (Wipf and Rixen, 2010).

Different *R. acris* populations grown under common conditions also responded differently in the onset of reproductive phenology, indicating genetic differentiation in the studied populations. However, the warmer and wetter treatment had opposite effects and plants from sites with later SMT had a faster phenological development than plants from early SMT site. This is consistent with other studies (Anderson et al., 2012; Carbognani et al., 2016; Kawai and Kudo, 2011) and might be due to a selective advantage flowering earlier in environments with a short growing season. Consistently, plants transplanted to warmer & wetter climate showed no such responses, suggesting that the opposite effects of temperature and precipitation change cancel each other out. In contrast to reproductive phenology, the leaf size of plants grown at the same site does not vary depending on origin, suggesting less differential selection and local adaptations in growth across sites. Growth has been suggested to be driven by other factors than climate, such as nutrient and water availability (Sedlacek et al., 2015), and especially in alpine plants, there might be trade-offs between growth and reproduction (Kudo, 1992; Molau, 1993; Theurillat and Schlussel, 2000).

Ranunculus acris had the ability to track variation in environmental conditions via phenotypic plasticity and at the same time showed clear indication of genetic differentiation on the phenological development, in accordance with other studies (Forrest and Miller-Rushing, 2010; Franks et al., 2014; Kawai and Kudo, 2011). Overall, the plastic response was stronger for phenology and growth, but not across all transplant treatments. For example, plants exposed to warmer climates reduced the pre-flowering period by approximately 25% compared to the origin-control plants, but also by 30% compared to destination-control plants. The combination of high plasticity and genetic response to warmer climate in high-elevation plants thus resulted in an enormous reduction in the pre-flowering period. This reduction is much larger than the difference in the pre-flowering time between the control

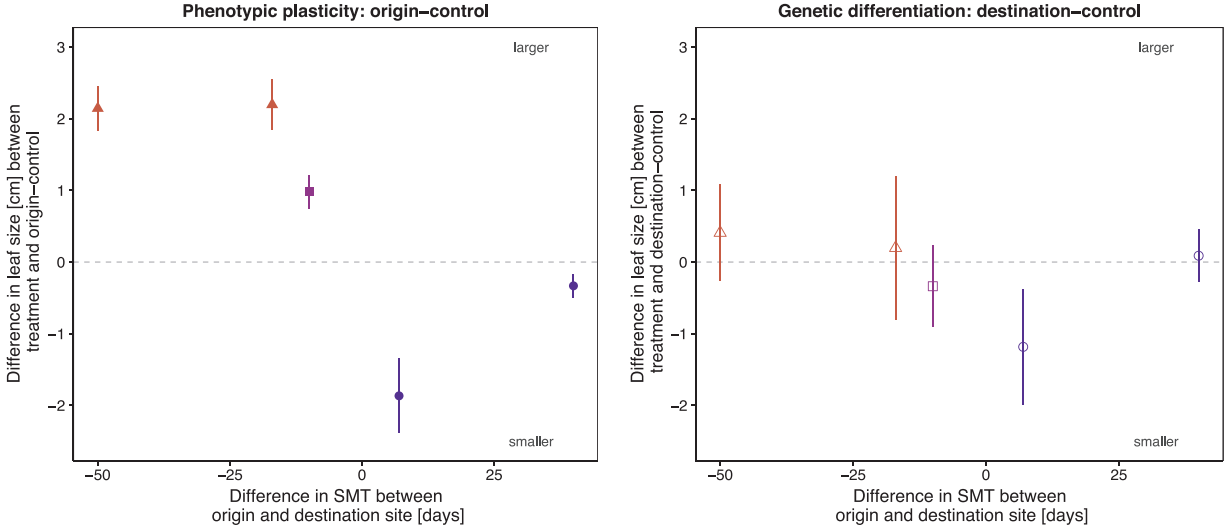
Phenotypic plasticity: origin–control
Genetic differentiation: destination–control
larger
smaller
Difference in leaf size [cm] between treatment and origin–control
Difference in leaf size [cm] between treatment and destination–control
Difference in SMT between origin and destination site [days]

Fig. 3. Plasticity and genetic difference in growth as the difference in leaf size ± 2SE in cm between treatment and origin-control (left) or destination-control (right) plants. The x-axis represents the difference in SMT between origin and destination site. The colours and symbols indicate the transplant treatments to: warmer (red triangle), wetter (blue circle) and warmer & wetter (purple square) climate. Points above/below the dashed grey line indicate larger/smaller leaf size in the transplanted plants compared to the origin-control (left) or destination-control (right) plants. Closed symbols indicate a significant difference between treatment and destination-control plants and open symbols no significant difference.

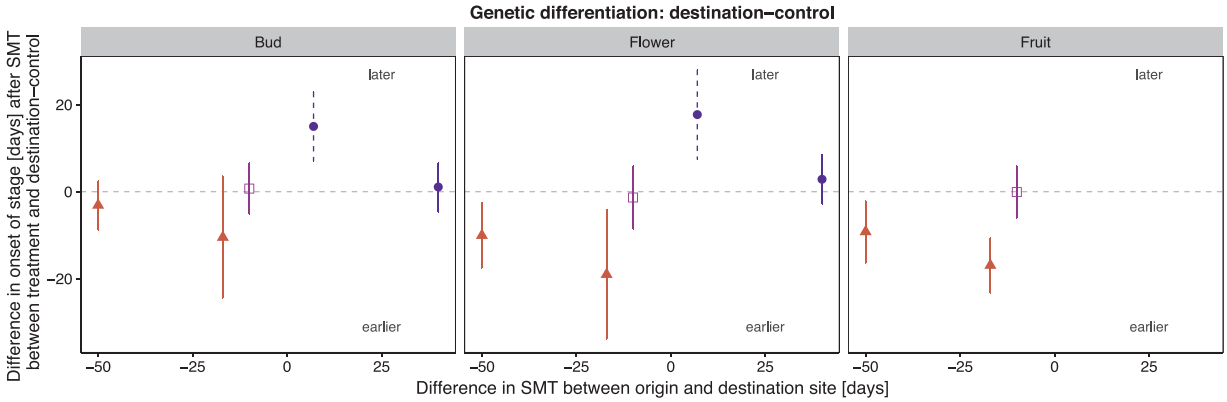

Fig. 4. Genetic difference in phenological development as the difference in onset of a phenological stage in days since SMT ± 2SE between treatment and destination-control site. The x-axis represents the difference in SMT between origin and destination site. The different stages are bud, flower and fruit. The colours and shapes indicate the transplant treatments to: warmer (red triangle), wetter (blue circle) and warmer & wetter (purple square) climate. Points below/above the dashed grey line indicate earlier/later days since SMT for onset in a stage in the transplanted plants compared to the destination-control plants. Closed symbols indicate a significant difference between treatment and destination-control plants and open symbols no significant difference. Dashed error bars indicate a sample size lower than 6 individuals.

plants at high- and low-elevation sites, suggesting that plants from high-elevation sites respond plastically by advancing their phenologies in response to increased temperatures, but also that they are genetically different and progress through the flowering phenology faster than the low-elevation plants. The combined effect is that their phenologies are strongly advanced compared to low-elevation populations in response to warming. However, we did not test the response of the low-elevation plants to warming.

In contrast, plants exposed to wetter climates started the onset of flowering 13% earlier than their origin-control plants, but were still 8% later than destination-control plants. Plants transplanted to warmer & wetter climates reduced the pre-flowering period by 17% compared to origin-control plants and flowered more or less at the same time as destination-control plants. For these two transplant treatments, the plastic response was considerably larger than the genetic response and generally corresponded to the shortened pre-flowering period between the control plants across sites. Overall, *R. acris* responded plastically to the climate treatments in all phenophases, with some exceptions.

4.2. Reproductive phenology and growth across climate contexts

Ranunculus acris showed similar within-population variation in plasticity in the onset of phenology (equally long error bars in Fig. 2) across different SMTs. We expected plants in harsher climate conditions, i.e. wet and high-elevation to be more genetically constrained to the extreme SMT in these sites and therefore exhibit less phenotypic plasticity (Frei et al., 2014; Gugger et al., 2015; Vitasse et al., 2013). Indeed, plants that were exposed to the most severe advance in SMT (i.e. transplanted from wet and high-elevation to wet and low-elevation), shifted the onset in phenology less compared to plants that experienced less severe advance in SMT (Fig. 2). Nine of the plants from wet and high-elevation site transplanted to warmer climate, managed to flower. But four of these did not advance the onset of phenology compared to the origin-control plants (error bar crosses zero line in Fig. 2), suggesting less plasticity in some genotypes of this population. Thus, plants from late SMT sites could be less plastic due to costs to phenotypic plasticity (Ernande and Dieckmann, 2004; Moran, 1992; van Tienderen and van der Toorn, 1991). Also, this population is likely to be close to the range edge of its distribution, which could explain the reduced plasticity. Overall, the largest magnitude in plant response did not correspond to the largest change in SMT the plant experienced, suggesting that plants can be constrained in their responses (see below).

Plants at high- and low-elevation sites also differed in how plastically they responded to delayed SMT. However, we had too few individuals from low-elevation sites to draw any conclusion on the difference in plasticity, which is likely to be due to the low sample size. The strong acceleration in the onset of phenology when SMT was strongly delayed (high-elevation plants) is possibly because later in the season temperatures are higher and speed up plant development (Domènech et al., 2016). Temperature in the first week after snowmelt was 1.6 °C higher in the high than low-elevation site, which could explain this acceleration in development.

Plants exposed to the warmer climate grew twice as tall than the origin-control plants, independent of how much advance in SMT they experienced. The plants exposed to earlier SMT, did not produce larger leaves even though their growing season was 50 days longer, possibly because there is a maximum leaf size that can be reached. Plants transplanted to wetter climate, had 13% shorter leaves at high-elevation and 40% at low-elevation compared to the origin-control plants. Plants at high-elevation generally had smaller leaves, and the negative effect of wetter climate therefore had a smaller effect on leaf size compared to the plants at low-elevation with on average larger leaves.

4.3. Responses to climate change in reproduction phenology

In this study, we were interested in how *R. acris* will respond to future climate, i.e. in a warmer and wetter world. We did not perform a true test of whether or not the species responses to different climates were adaptive, which would require reciprocal transplants experiments (Blanquart et al., 2013; Kawecki, 2008; Merilä and Hendry, 2014). We used individuals grown in their original habitat and can therefore not completely rule out confounding maternal carry-over effect on local adaptation (Kawecki and Ebert, 2004). Local plants did not produce more fruits (data not shown) compared to non-local plants, suggesting no evidence that *R. acris* is locally adapted, however, this interpretation should be taken with caution. *R. acris* plants were taller and produced more fruits when grown at low-elevation, irrespective of origin (controls, warmer and warmer & wetter transplant treatment), suggesting no maladaptation to the predicted future climate in this region. On the contrary, the highly plastic response in the onset of all phenophases, allowed *R. acris* to profit from the longer growing season after advanced SMT. The genetic constraint to start the phenological development earlier did not result in fitness loss, at least not in the year of this study. However, we do not know if early flowering in a less snow-rich year could confer a disadvantage, for example due to frost events (Inouye, 2008; Thomson, 2010). The study sites had a low replication and we can therefore not rule out that some of the effects we saw were due to other abiotic (soil) and biotic factors we did not control for in this experiment. However, the sites were chosen to be as similar as possible to reduce such effects.

All *R. acris* plants drastically reduced the fruit production when grown in the wet high-elevation site in the year of the study, due to late snowmelt. Although the present study was carried out during an extremely snow rich year, further and rapid adaptation might be necessary in wet alpine sites for species to persist (Anderson et al., 2012). Even populations of widespread species may be more vulnerable to extreme climate events (i.e. a snow-rich year) than inferred from their distribution alone. Finally, the interaction of increased precipitation and warmer temperatures are complex and could also result in a delayed SMT, and lead to later flowering in the season and at warmer temperatures. This could accelerate the phenological development and lead to probably much more synchronized flowering across different species within a plant community (Carbognani et al., 2016). Synchrony in flowering among species can attract pollinators and increase reproduction (Moeller and Geber, 2005; Totland, 1993), but may also lead to increased pollen limitation (García-Camacho and Totland, 2009).

Author contribution

ND collected and analysed data and wrote the manuscript, **AP** commented on the manuscript, **MC** analysed data and wrote the manuscript, **VV** designed the experiment and commented on the manuscript and **AHH** designed the experiment, collected data and wrote the manuscript.

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Appendix A

Number of individual plants per origin and transplant site.

Transplant site	Origin	Number of plants
High-elevation dry	High-elevation dry	30
Low-elevation dry	High-elevation dry	40
	Low-elevation dry	10
High-elevation wet	High-elevation dry	40
	High-elevation wet	30
Low-elevation wet	High-elevation dry	40
	Low-elevation dry	9
	High-elevation wet	40
	Low-elevation wet	30

Appendix B

Plasticity of reproductive phenology (in days after SMT) and leaf size (in cm) between origin-control plants and transplants to a) warmer, b) wetter and c) warmer & wetter transplant treatment. Analysis of deviance tables on the effects of transplant treatment, precipitation level or temperature level and their interactions on the onset of bud, flower and fruit stage between stages and leaf size of *Ranunculus acris*. Values are χ^2. (Significance codes: *P*-value < 0.001 '***'; < 0.01 '**'; < 0.05 '*'; > 0.05 ' ').

Compare to origin-control	Bud	Flower	Fruit	Leaf size
a) Warmer transplant treatment				
Warmer treatment	45.65 ***	42.36 ***	12.17 ***	83.95 ***
Precipitation level	56.92 ***	55.66 ***	36.22 ***	3.68
Warmer × Precipitation level	1.73	0.002	1.01	1.27
b) Wetter transplant treatment				
Wetter treatment	62.81 ***	19.51 ***	NA	14.73 ***
Temperature level	3.66	7.99 **		36.99 ***
Wetter × Temperature level	10.76 **	2.75		3.86 *
c) Warmer & wetter transplant treatment				
Warmer & wetter treatment	114.73 ***	50.79 ***	24.18 ***	18.38 ***

Appendix C

Genetic response of reproductive phenology (in days after SMT) and leaf size (in cm) between destination-control plants and transplants to a) warmer, b) wetter and c) warmer & wetter transplant treatment. Analysis of deviance tables on the effects of treatment, precipitation level or temperature level and their interactions on the onset of bud, flower and fruit stage between stages and leaf size of *Ranunculus acris*. Values are χ^2. (Significance codes: *P*-value < 0.001 '***'; < 0.01 '**'; < 0.05 '*'; > 0.05 ' ').

Compare to destination-control	Bud	Flower	Fruit	Leaf size
a) Warmer transplant treatment				
Warmer treatment	8.70 **	28.38 ***	16.99 ***	1.23
Precipitation level	49.23 ***	41.93 ***	32.11 ***	3.05
Warmer × Precipitation level	0.73	0.32	0.34	0.19
b) Wetter transplant treatment				
Wetter treatment	8.86 **	7.69 **	NA	2.17
Temperature level	7.58 **	4.68 *		39.37 ***
Wetter × Temperature level	6.33 *	5.22 *		5.87 *
c) Warmer & wetter transplant treatment				
Warmer & wetter treatment	0.12	0.25	< 0.001	1.44

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